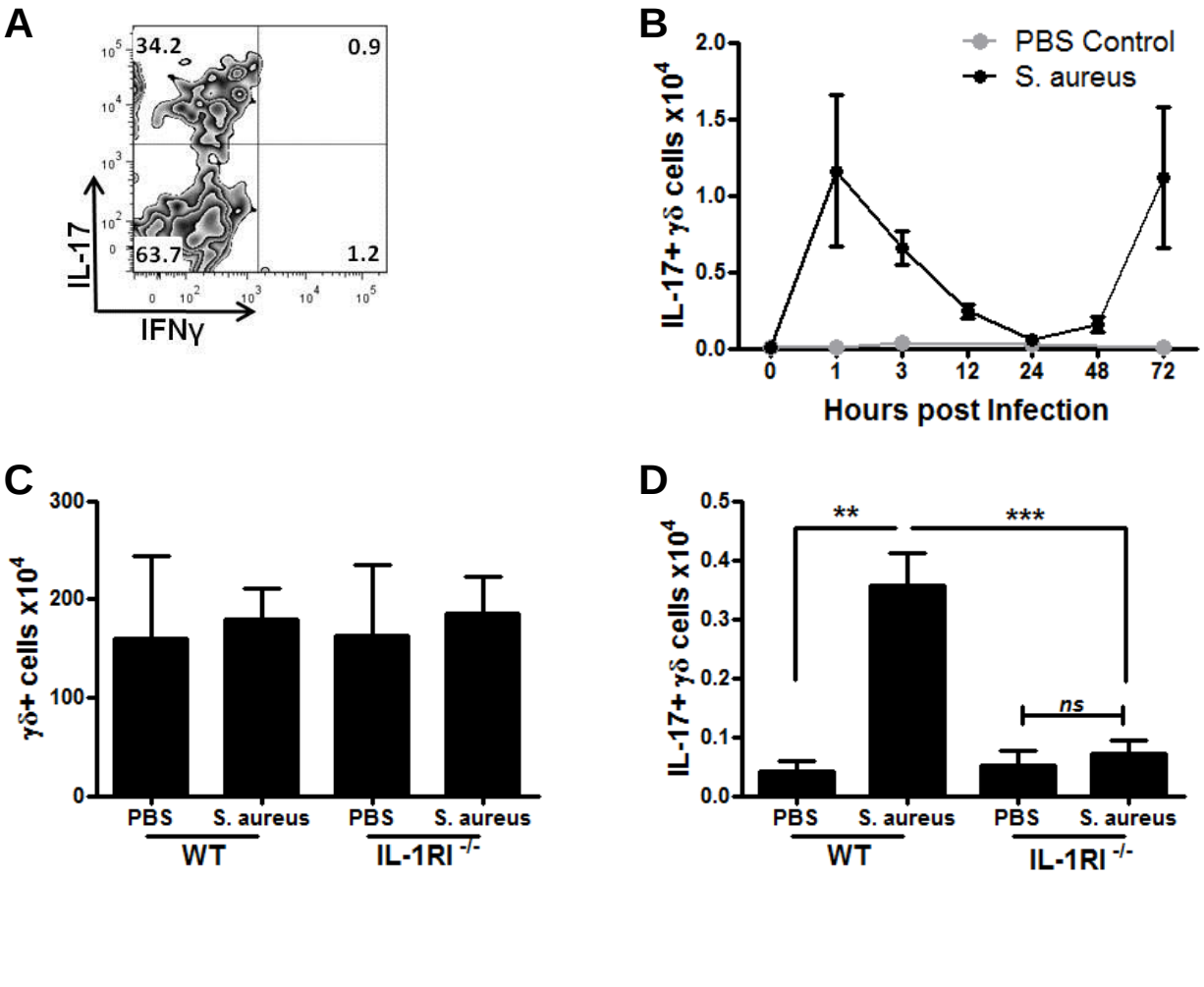


F ***Mus musculus*** **T cell receptor gamma chain (Tcrg) on chromosome 13**
NCBI Reference Sequence: NG_007033.1
(gi|159120306:12827-12920, 13077-13388)

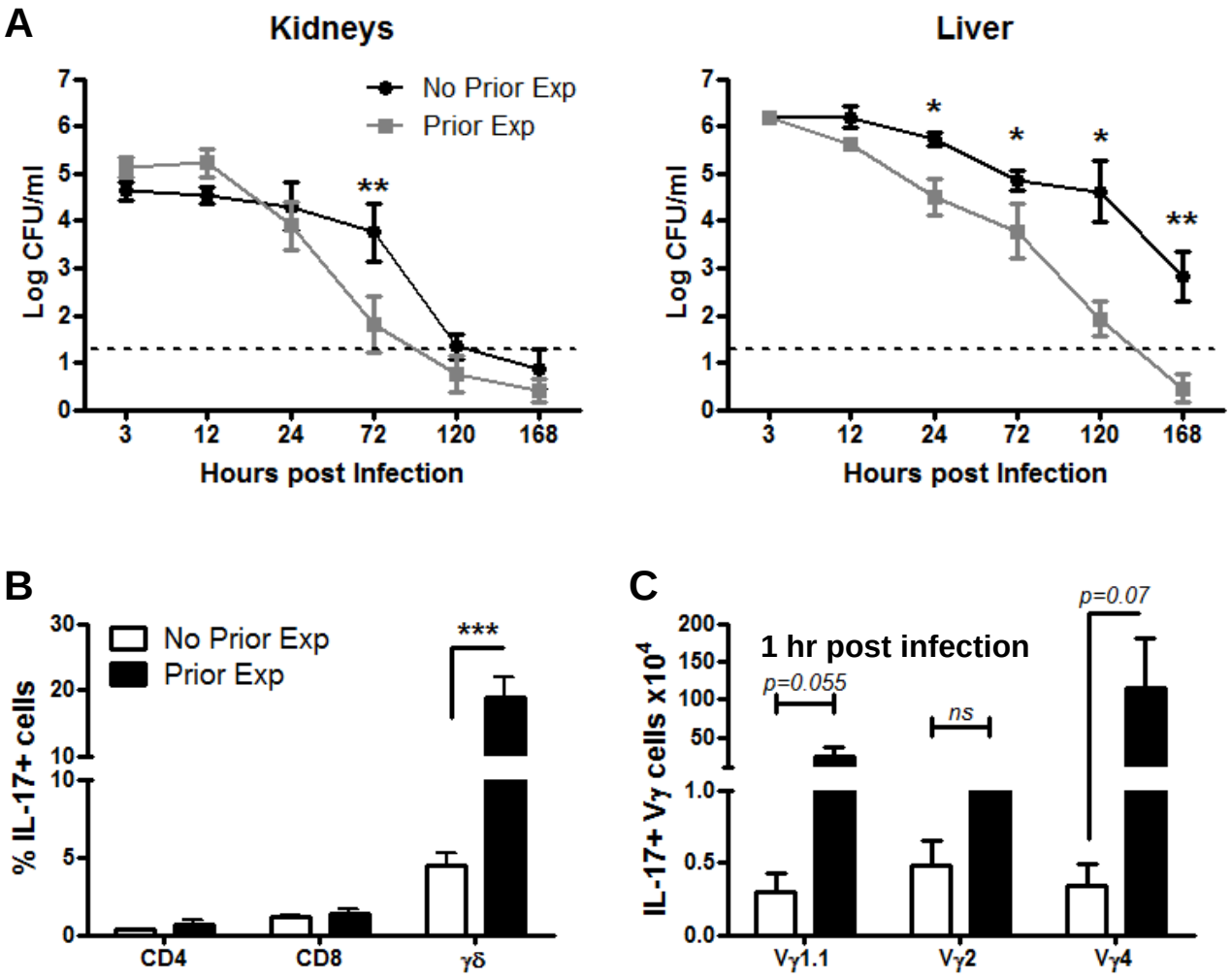
Alignment Score: 93.1%

```
Reference: AVSRHLWGHMSSRGKEIRLFSNVKKQVFRSPMHTYTGTKRSQASVSKECCVVLQKKTL
Vy4      : AVSRHLWGHMSSRGKEIRLFSNVKKQVFRSPMHTYTGTKRSQASVSKECCVVLKRKHV
           ************************************************::* :
```

Supp Fig 1

Supp Figure 1: γδ T cell accumulation in the peritoneal cavity during S. *aureus* infection

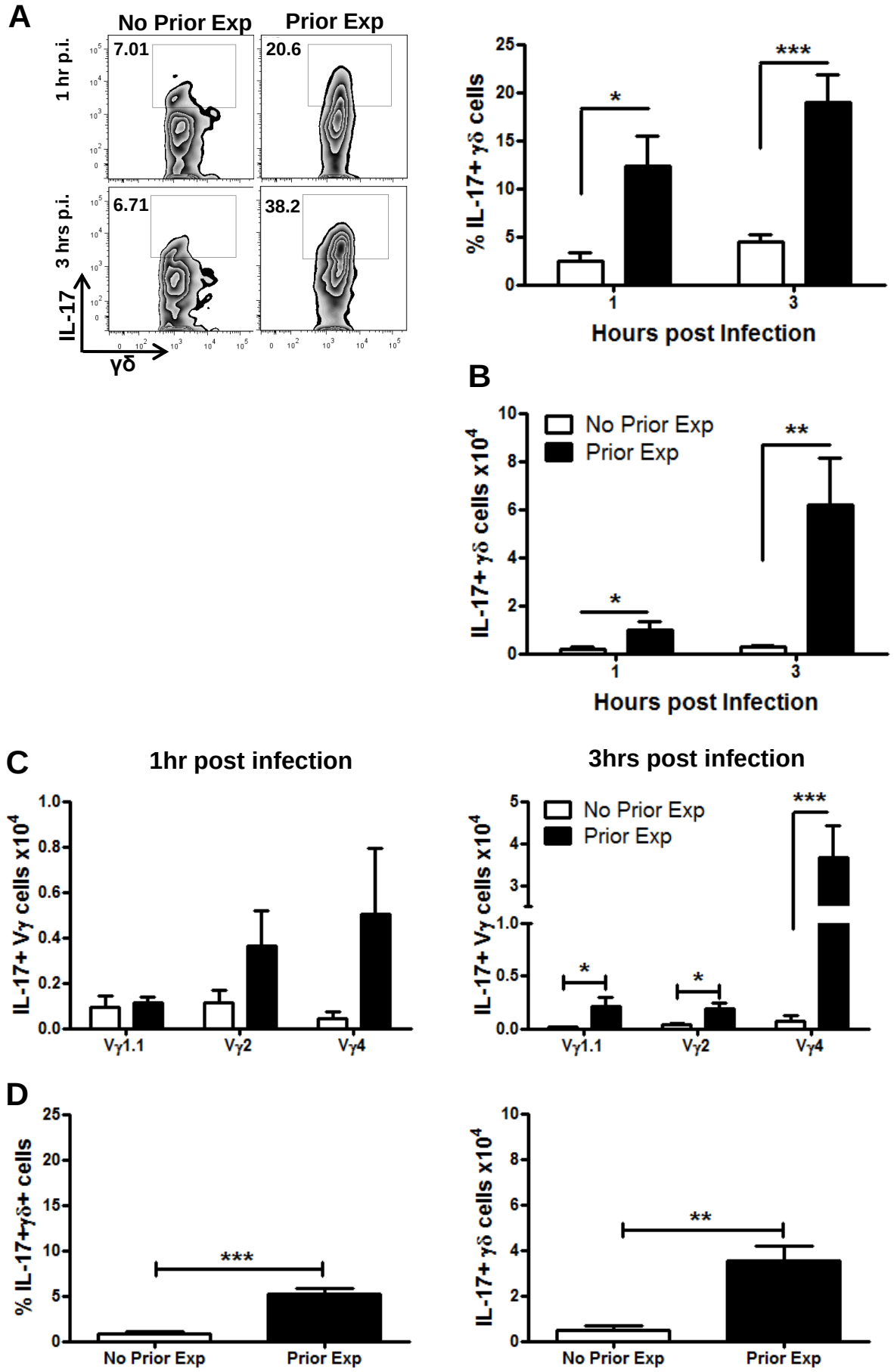
Mice were infected with *S. aureus* ($5x10^8$ CFU) via i.p. injection. At the indicated time points post-infection, PECs were cultured with Brefeldin A, but not PMA and ionomycin, stained for surface CD3 and γδTCR, and intracellular IL-17 and IFNγ, and analysed by flow cytometry. (A) Representative FACS plot at 3 h post-infection. (B) The absolute numbers of IL-17-producing γδ T cells in the peritoneal cavity are expressed as mean ± SEM of n=5 mice/group. Data are representative of 2 independent experiments. WT and IL-1RI$^{-/-}$ mice were infected with *S. aureus* ($5x10^8$ CFU) via i.p. injection and γδ T cell recruitment (C) and IL-17 production (D) assessed by flow cytometry at 3 h post-infection. Results are expressed as mean ± SEM of n=10 mice/ group. Data are representative of 2 independent experiments. Mice were infected with *S. aureus* ($5x10^8$ CFU) via i.p. injection. PECs were harvested from mice at 3 h post-infection and Vγ1.1$^-$Vγ2$^-$ cells purified by FACS. RNA was extracted from the purified Vγ1.1$^-$Vγ2$^-$ cells, reverse transcribed into cDNA and each Vγ gene amplified by PCR (E). Amplified DNA was excised from the gel, translated and aligned with the Vγ4 sequence (NCBI reference gene (NG_007033.1)) (F).



Supp Figure 2: Prior exposure to *S. aureus* leads to expansion of IL-17$^+$ Vγ4 T cells and protects against dissemination of bacteria during subsequent infection.

Groups of mice were exposed to *S. aureus* (5x10^8 CFU) via i.p. injections on d 0, 7 and 14, and allowed to recover for 21 d. Previously exposed mice were then re-challenged with *S. aureus* (5x10^8 CFU) on day 35, as were a control group of naive mice. At the indicated time points following challenge, bacterial burden was assessed in the kidneys and liver (A). Results expressed as log CFU/ml of n=12-15 mice/ group. At 3 h post-infection, MLN cells were cultured with Brefeldin A, but not PMA and ionomycin, and stained for surface CD3, CD4, CD8 and γδTCR, and intracellular IL-17, and analysed by flow cytometry (B). At 1 h post infection isolated PECs were cultured with Brefeldin A, but not PMA and ionomycin, and IL-17 production by individual γδ T cell subsets analysed by flow cytometry (C). Results expressed as mean ± SEM of n=12 mice/ group. *p<0.05, **p<0.005, ***p<0.001. Data represent 4 independent experiments.

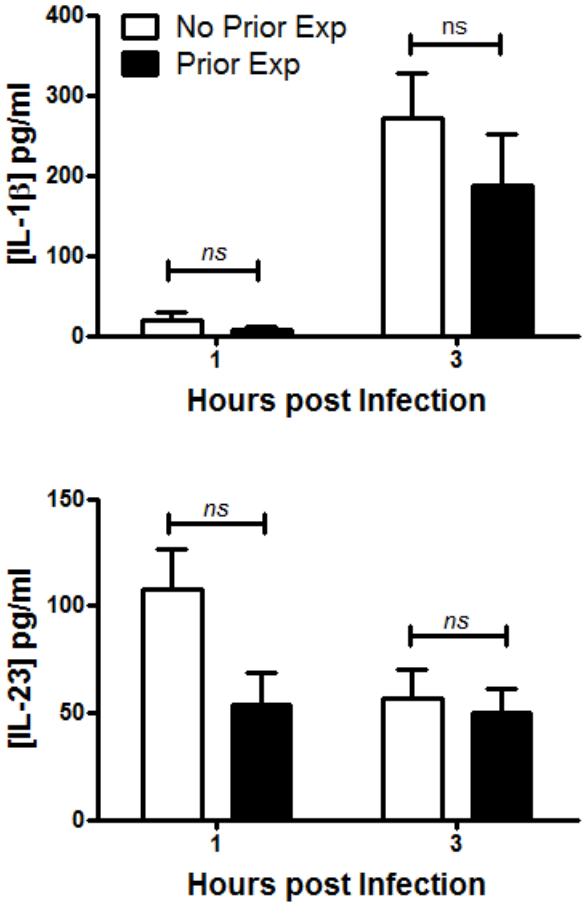
Supp Fig 2


A
No Prior Exp
Prior Exp
1 hr p.i.
3 hrs p.i.
7.01
20.6
6.71
38.2
IL-17
γδ
% IL-17+ γδ cells
Hours post Infection
B
No Prior Exp
Prior Exp
IL-17+ γδ cells x10^4
Hours post Infection
C
1hr post infection
3hrs post infection
IL-17+ Vγ cells x10^4
Vγ1.1
Vγ2
Vγ4
D
% IL-17+γδ+ cells
IL-17+ γδ cells x10^4

Supp Fig 3

Supp Figure 3: Prior exposure to *S. aureus* results in the expansion of IL-17-producing Vγ1.1^{+} and Vγ4^{+} T cells in the MLN and spleen upon re-challenge.

Groups of mice were exposed to *S. aureus* (5x10^{8} CFU) via i.p. injections on d 0, 7 and 14. Previously exposed mice were then re-challenged with *S. aureus* (5x10^{8} CFU) on d 35, as were a control group of naive mice. At 1 and 3 h post-challenge, MLN cells were cultured with Brefeldin A, but not PMA and ionomycin, stained for surface CD3, γδTCR, Vγ1.1, Vγ2 and Vγ3, and intracellular IL-17, and analysed by flow cytometry. Results expressed as mean ± SEM of n=9 mice/ group, with representative FACS plots (A & B). IL-17 expression by individual Vγ subsets amongst total γδ T cells in the MLN was also assessed at 1 and 3 h post-challenge (C). Results expressed as mean ± SEM of n=9-12 mice/ group. At 3 h post-challenge, spleen cells were cultured with Brefeldin A, but not PMA and ionomycin, stained for surface CD3, γδTCR and intracellular IL-17, and analysed by flow cytometry (D). Results expressed as mean ± SEM of n=5 mice/ group. *$p<0.05$, **$p<0.005$, ***$p<0.001$. Data represent 2-3 independent experiments.



Supp Figure 4: Elevated IL-17 production by γδ T cells upon re-challenge is not associated with increased IL-1β or IL-23 secretion in the peritoneal cavity .

Groups of mice were exposed to *S. aureus* ($5x10^8$ CFU) via i.p. injections on d 0, 7 and 14. Previously exposed mice were then re-challenged with *S. aureus* ($5x10^8$ CFU) on d 35, as were a control group of naive mice. At 1 and 3 h post challenge secreted IL-1β and IL-23 in the peritoneal fluid was measured by ELISA.

Supp Fig 4